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Original Article

Determination and Diversity of Bovine Coccidia in Zabol, East of Iran

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ABSTRACT

The present study was designed to investigate the prevalence of Eimeria spp. in Zabol, east of Iran. The samples were examined microscopically for the presence of oocysts from February 2015 to December 2016. Out of 196 cattle, 124 (63.26%) were identified to be positive for Eimeria species. Eight species of Eimeria were found as follows: *E. bovis* (42.54%), *E. zuernii* (38.67%), *E. subspherica* (5.52%), *E. brasiliensis* (4.97%), *E. ellipsoidalis* (4.41%), *E. cylindrical* (1.65%), *E. pellita* (1.65%), and *E. wyomingensis* (0.55%). Multiple infections with two or three species were identified in 56 (45.16%) and 2 (1.61%) cases, respectively. The infection rate was observed to be higher in calves (75%) as compared to adult cattle (59.02%). The prevalence of Eimeria was greater in female cattle (67.94%). The highest prevalence and mean number of oocysts were observed in winter (69%) and autumn (209.09), respectively. All the faecal samples had an oocysts per gram of feces (OPG) less than 1000. The prevalence of infection and mean OPG were directly correlated with rainfall and relative humidity and inversely correlated with temperature. Considering the high prevalence of bovine coccidiosis in Zabol, increasing awareness of farmers and veterinarians regarding the economic importance of bovine coccidiosis should be regarded as an important factor in preventing and controlling the infection.

Keywords: Prevalence, Cattle, *Eimeria spp.*, Risk factors, Iran

La détermination et la diversité de coccidies bovines à Zabol dans l'Est iranien

Résumé: L'objectif de cette étude était d'évaluer la prévalence des espèces d'Eimerias à Zabol dans l'Est iranien. Les échantillons ont été examinés au microscope de Février 2015 à Décembre 2016 afin de déterminer la présence d'oocystes. Sur 196 bovins, 124 (63,26%) étaient positifs pour les espèces d'Eimeria. Les huit espèces identifiées étaient les suivantes : *E. bovis* (42,54%), *E. zuernii* (38,67%), *E. subspherica* (5,52%), *E. brasiliensis* (4,97%), *E. ellipsoidalis* (4,41%), *E. cylindrique* (1,65%), *E. pellita* (1,65%) et *E. wyomingensis* (0,55%). Les taux d'infections multiples causées par deux ou trois espèces étaient respectivement de 56 (45,16%) et 2 (1,61%). La prévalence des infections était plus élevée chez les bovins (75,00%) que chez les adultes (59,02%). Les bovins femelles étaient les plus infectées et constituaient 67,94% des cas détectés. La prévalence la plus élevée et le nombre moyen d'oocystes ont été respectivement observés en hiver (69,00%) et en automne 209,098. Tous les échantillons fécaux avaient un OPG (oocystes par gramme de fèces) inférieur à 1000. La prévalence de l'infection et l'OPG moyen corrélaient directement aux taux de précipitations et

d'humidité relative, alors qu'il montrait une corrélation inverse avec la température. Etant donnée la forte prévalence de la coccidiose bovine à Zabol, il faut considérer la sensibilisation des agriculteurs et des vétérinaires à l'importance économique de la coccidiose bovine comme un facteur important dans la prévention et le contrôle de l'infection.
Mots-clés: Prévalence, Bovins, *Eimeria spp.*, facteurs de risque, Iran

INTRODUCTION

Bovine coccidiosis is an infectious disease of the cattle that is of great economic importance all over the world. Twenty-one species based on morphological and physiological criteria are identified to infect bovines, of which 15 are reported in Iran as follows: *E. bovis*, *E. zuernii*, *E. auburnensis*, *E. canadensis*, *E. ellipsoidalis*, *E. subspherica*, *E. cylindrica*, *E. alabamensis*, *E. wyomingensis*, *E. bukidnonensis*, *E. illinoisensis*, *E. pellita*, *E. mundargi*, *E. bombayensis*, and *E. brasilensis* (Farkas et al., 2007; Yakhchali and Gholami, 2008; Bahrami and Alborzi, 2013; Heidari et al., 2014). All the species belong to the genus Eimeria, which infects cattle. This protozoa can cause infection in animals at any age, and clinical coccidiosis occurs mainly in cattle (Farkas et al., 2007). E. bovis and E. zuerniiare are considered to have high pathogenicity, while infection with most other species may induce mild disease in cattle (Koutny et al., 2012). Clinical symptoms, including diarrhea, dysentery, tenesmus, reduced weight gain, weakness, and listlessness are observed in heavy infections (Mundt et al., 2005). Sub-clinically infected animals appear normal outwardly, but they do not have enough feed consumption, feed conversion, and growth performance (Cicek et al., 2007). The diagnosis of coccidiosis should be based on the observation of clinical signs, the abundance of important oocyst species, and the presence of intestinal lesions at necropsy. Oocysts in the acute stage of coccidiosis are not observed in fecal samples, but the presence of oocysts and determination of oocysts per gram of feces (OPG) in fecal examination are important factors in confirmation of clinical coccidiosis (Heidari et al., 2014). Reducing infection with Eimeria spp. is associated with general hygiene and avoidance of overcrowding and stress (Mitchell et al., 2012). However, there is little information regarding the risk factors associated with Eimeria spp. infection in Iran. We carried out this study to determine the prevalence of Eimeria and its associated risk factors in cattle of Zabol, east of Iran.

MATERIALS AND METHODS

Study area. This study was carried out in Zabol during February 2015-December 2016. Zabol is located in the east of Iran and north of Sistan and Baluchestan Province, between 31°13′ degree north latitudes and 61°29′ degree longitudes. Zabol is 489.2 m above sea level. Zabol, from the north and east, borders with Afghanistan and is limited from the west and northwest to dry deserts. During the past 20 years, the maximum temperature in this area ranged from 22 to 49, while the minimum temperature has been -8. The average annual rainfall and humidity in Zabol are reported at around 59 ml and 40%, respectively (Radfar et al., 2012).

Sample collection. Based on two-stage cluster random sampling, the number of primary units were defined using coordinates of a map grid (four farms in north, south, east, and west of Zabol) and secondary units (animals in each selected region) were sampled using the formulae described by Thrusfield (2013). One hundred and ninety-six cattle were evaluated in the current study. The animals were reared under traditional system and fed on the ground. The animals were classified into two age groups. The age ranges of the calves and adult cattle were 1-3 and 3-7 years, respectively. No clinical signs were observed among the infected cattle in the current study. Fecal samples were collected from the rectum of 196 cattle using

examination gloves, or in some cases, freshly excreted feces were picked up from the ground and transferred into plastic bottles. Sex, age, and date of sampling of each animal were labelled on the plastic bottles and stored under refrigerated conditions until processing. All the samples were classified based on their consistency as normal or diarrheic feces (liquid or semi-liquid feces).

Faecal examination. Three grams of each sample was analyzed for the presence of oocysts by direct flotation in saturated sucrose solution. The positive samples were examined using modified McMaster fecal egg counting technique and Sheather's sugar flotation solution to estimate OPG (Koutny et al., 2012). For the sporulation of oocysts, the remaining materials from the McMaster slides were separately transferred into glass bottles containing 2.5% potassium dichromate solution. The glass bottles were kept at laboratory temperature during two weeks as described by (Pyziel and Demiaszkiewicz, 2013). *Eimeria* spp. were identified using the morphological features of the oocysts, including length, width, their range and ratio, micropyle, micropyle width, micropyle cap, micropyle cap width, micropyle cap depth, polar granule, and oocyst residuum, as well as sporocyst characteristics consisting of length, width, their range and ratio, Stieda body, sub-Stieda body, para-Stieda body, sporocyst residuum, and refractile bodies according to Duszynski and Wilber (1997).

Meteorological data. Daily meteorological data such as rainfall, temperature, and relative humidity were provided by the Meteorological Department of Zabol. The average temperature, relative humidity, and total rainfall were calculated for each season separately. The minimum relative humidity and rainfall were recorded 13% and 1 mm in summer, respectively, while the maximum rainfall (33 mm) and relative humidity (39%) were observed in winter as compared to other seasons. The lowest and highest temperatures were recorded 7.8 °C and 37.8 °C in winter and summer, respectively.

Statistical analysis. For the qualitative data, descriptive statistics were used in SPSS, version 19. Logistic regression with 95% confidence interval (95% CI) was used to survey the effect of risk factors on the prevalence of infection. Pearson correlation coefficient was run to determine the correlation of climatic parameters with the prevalence of infection and mean OPG. P-value less than 0.05 was considered statistically significant.

RESULTS

Out of the examined 196 faecal samples, 124 (63.26%) were positive for coccidial oocysts. We found eight species of *Eimeria*, including *E. bovis, E. zuernii*, *E. subspherica*, *E. brasiliensis*, *E. ellipsoidalis*, *E. cylindrical*, *E.* pellita, and *E. wyomingensis*. *E. bovis* was the most prevalent species (42.54%), while *E. wyomingensis* was the rarest (0.55%). Mixed infections with two or three species were found in 56 (45.16%) and 2 (1.61%) cases, respectively. However, most animals were infected to one species (53.22%). Mixed infections with two species were observed mainly in calves (56.41%; Table 1). Maximum mean excerted OPG was recorded 209.98 in autumn. Association of climatic parameters with mean OPG is presented in Figure 1. Generally, calves showed higher mean OPG than adult cattle. The mean OPG values of the samples from adult cattle and calves for each season are exhibited in Figure 2. The analysis of the data by Pearson correlation coefficient revealed a positive correlation between prevalence and mean OPG and relative humidity and rainfall in Zabol, while temperature was inversely correlated with prevalence and mean OPG in our study (P<0.05).

Associated risk factors. It was found that 75% of the samples were calves, while 59.02% were adult cattle (Table 2). There was a significant association between age and infection status (P=0.043; OR=2.082; Table 2). Eimeria infection was more prevalent in females (67.94%) as compared to males (60.16%), but no significant difference was observed in this regard (P=0.270; OR=1.403; Table 2). The highest prevalence

Table 1. The number of coccidia species present in individual positive faecal samples

Age class	n	Number of species in samples (%)		
		1	2	3
Calves	39	17 (43.58)	22 (56.41)	0 (0)
Adult cattle	85	49 (57.64)	34 (40.00)	2 (2.35)
Total	124	66 (53.22)	56 (45.16)	2 (1.61)

Table 2. The prevalence of Eimeria and associated risk factors in cattle of Zabol, east of Iran

Associated determinants	Variables	Prevalence (n/N) [a]	Odds ratio	95 % CI for OR	P value [b]
Age	Calves	75.00 % (39/52)	1	-	-
	Adults cattle	59.02 % (85/144)	2.082	1.024-4.236	0.043
Sex	Male	60.16 % (71/118)	1	-	-
	Female	67.94 % (53/78)	1.403	0.769-2.561	0.270
Season	Winter	69.00 % (29/42)	1	-	-
	Spring	66.00 % (33/50)	0.542	0.344-1.963	0.157
	Summer	54.71 % (29/53)	0.822	0.232-1.266	0.659
	Autumn	64.70 % (33/51)	0.870	0.362-2.093	0.756

[a] Prevalence (%) = n/N×100
[b]*P*-value, *P*< 0.05 was considered as statistically significant
n: number of animals infected; N, total number of animals examined

was observed during winter (69.00%; Table 2, Figure 3). The relationship between rainfall, relative humidity, temperature, and prevalence of *Eimeria* is presented in Figure 3.

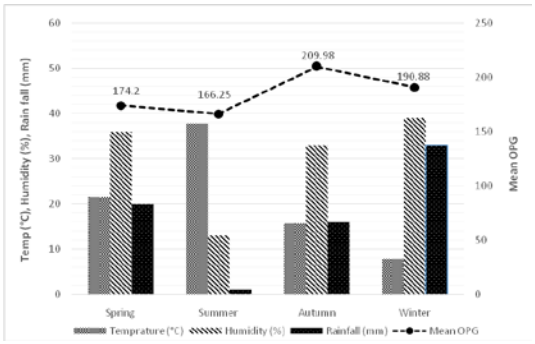

Figure 1. Association of climatic parameters with mean oocysts per gram of feces exerted in cattle of Zabol

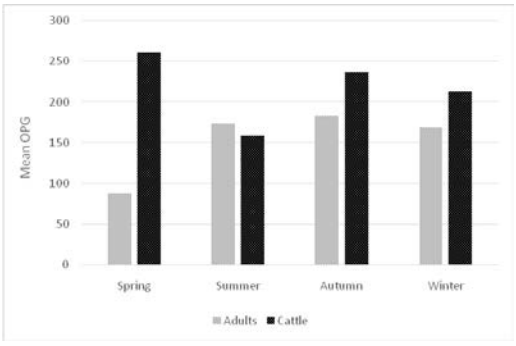

Figure 2. Mean oocysts per gram of feces shed from calves and adult cattle in each season

DISCUSSION

The current study showed the presence of bovine *Eimeria* species in calves and adult cattle in Zabol, east of Iran. The overall prevalence of *Eimeria* spp. was 63.26%, which is lower than the previous report (70.5%) in west of Iran by Heidari and Jamal (2014).

In other studies, its prevalence rates were reported 50%, 21.3%, and 8.25% in East-Azerbaijan, Kurdistan, and Hamadan provinces of Iran, respectively

(Yakhchali and Zareei, 2008; Davoudi et al., 2011; Heidari et al., 2014). In other countries, the prevalence rates of *Eimeria* infection in cattle were reported 82.28% in the coastal plain area of Georgia (the USA) by Ernst et al. (1987), 47.09% in Pakistan by Rehman et al. (2011), 33.3% in Brazil by Almeida et al. (2011), and 33% in Hungary by Farkas et al. (2007). Several factors can account for the difference in infection rate among various countries such as the number of ingested oocysts, the presence of a concurrent microbial infection, weather conditions (temperature, relative humidity, and rainfall), management of the farms, and the functional level of protective immunity (Heidari and Jamal, 2014). All the species identified in the present study have been previously reported in cattle in Iran. In this study, *E. bovis* and *E. zuernii* were the most prevalent species of Eimeria, which is in agreement with the findings of Koutny et al. (2012), Pyziel et al. (2014), and Almeida et al. (2011).

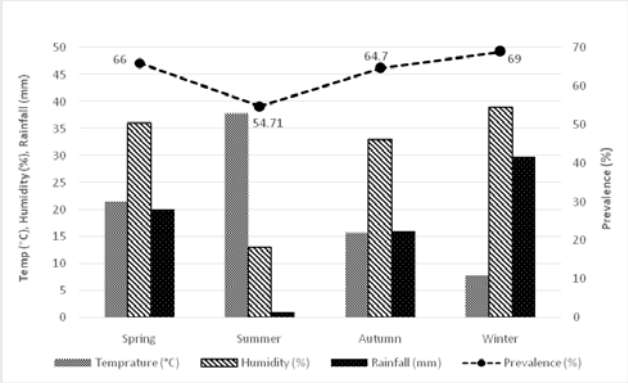

Figure 3. Association of climatic parameters with the prevalence of Eimeria in cattle of Zabol

Infection rate in calves was significantly higher than adult cattle. The higher prevalence in calves may be related to their immature immunity against *Eimeria* infection. In contrast, adult cattle have higher levels of protective immunity due to previous exposure, and therefore, are more resistant to following reinfections. In our study, the infection rate was observed to be higher in females as compared to males. There was no significant association between infection and animals' sex; accordingly, both sexes have an almost equal chance of *Eimeria* spp. infection. However, slightly higher prevalence in females could be attributed to the various hormones such as estrogen and progesterone that have immunosuppressive effects and considerably increase during pregnancy (Zuk and McKean, 1996). Heidari et al. (2014) reported similar findings. Waruiru et al. (2000) recorded that the prevalence of *Eimeria* infections in the wet seasons in cattle was significantly higher than the dry seasons. Duncan (1959) proposed that dryness was an important factor in killing oocysts. In our study, Pearson correlation coefficient showed a direct correlation between mean OPG and prevalence of infection and rainfall and humidity and an inverse correlation with temperature. In summer, the oocysts in closed soil die even faster than in winter and spring because of the high temperature and low humidity (Kheysin and Todd, 2013). In the current study, the highest mean OPG and the prevalence of Eimeria infection were found to be 209.98 and 69.00% in autumn and winter, respectively, indicating that optimum temperature (15.7°C) and relative humidity (33%) in autumn lead to sporulation of oocysts and increased *Eimeria* spp. infection in winter. In line with the study by Pyziel et al. (2014), we found higher mixed-species infections with the predominance of two species in calves (56.41%) as compared to adult cattle (40%). In our study, most faecal samples had low OPG, suggesting that the animals developed sub-clinical form of the infection. However, the sub-clinical infections play an important role in economic losses.

In conclusion, regarding the economic importance of bovine coccidiosis in reducing feed consumption, feed conversion, and growth performance, especially on larger farms and in areas with higher cattle density, monitoring and controlling the infection should be considered to prevent negative impacts on livestock production and health. The study of survival of oocysts on various types of pastures and under rigid external environmental conditions enables us to identify the definite periods of pasture change as a valuable

measure in the prophylaxis of coccidiosis. Due to the high prevalence of bovine coccidiosis in Zabol, promoting awareness of farmers and veterinarians concerning the economic importance and associated risk factors for bovine coccidiosis can play a pivotal role in the prevention and control of the disease.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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